BIOLOGICAL MONITORING PROGRAM

QUALITY ASSURANCE PROJECT PLAN FOR

WADEABLE STREAMS AND RIVERS

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Date: April 24, 2006

Group A: Project Management Elements

A1 – Title and Approval Sheet

Virginia Department of Environmental Quality
Biological Monitoring of Virginia
Quality Assurance Project Plan for
Wadeable Streams and Rivers

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A2 – Table of Contents

Element/ Section

Appendix B Standard Operating Procedures
- i. Methods for Benthic Macroinvertebrate Collections in Cobble Substrate (single habitat)
- ii. Methods for Multi-habitat Benthic Macroinvertebrate Collections
- iii. Methods for Habitat Assessment for Streams
- iv. Methods for Laboratory Sorting and Subsampling of Benthic Macroinvertebrate Samples

Appendix C Assessment Determination
- i. Rapid Bioassessment Protocols (RBP II): Metric scoring criteria, assessment categories, and metric definitions
- ii. The Coastal Plain Macroinvertebrate Index (CPMI): Metric scoring criteria, assessment categories, and metric definitions

Appendix D Data Sheets
- i. Benthic Macroinvertebrate Field Data Sheet
- ii. Benthic Macroinvertebrate Laboratory Bench Sheet

Appendix E Regional Biologist Assessment Checklist

A3 – Distribution List

Name	Organization
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Ellen Gilinsky	Virginia Department of Environmental Quality
Jean Gregory	Virginia Department of Environmental Quality
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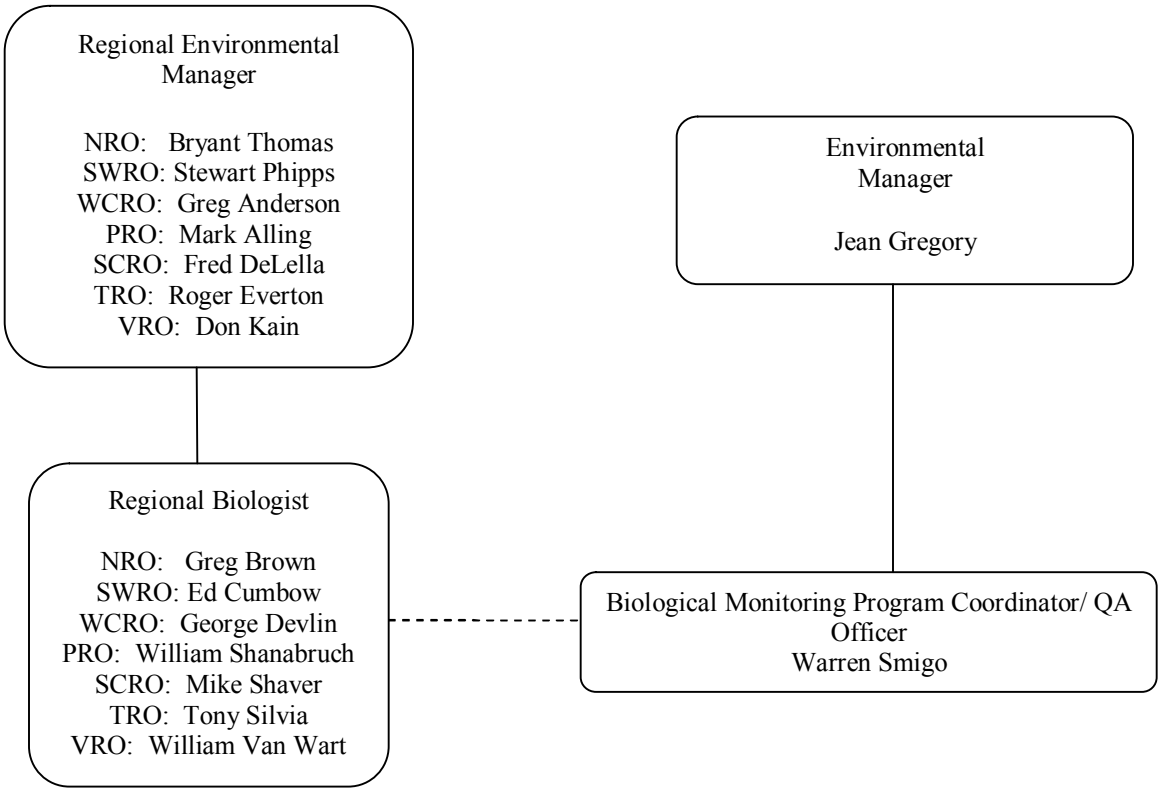
Other staff from Virginia Department of Environmental Quality as appropriate.

A4 – Project/ Task Organization



VADEQ's freshwater biological monitoring program is conducted out of seven regional offices located throughout Virginia. These offices are located in Abington (Southwest Regional Office), Roanoke (West Central Regional Office), Lynchburg (South Central Regional Office), Harrisonburg (Valley Regional Office), Woodbridge (Northern Regional Office), Glen Allen (Piedmont Regional Office), and Virginia Beach (Tidewater Regional Office). Each regional office is staffed with a regional biologist under the direction of the regional environmental manager. The biological monitoring program coordinator in the Central Office of VADEQ in Richmond is responsible for the coordination of the biological monitoring program and also serves as the program QA officer. The program coordinator is under the direction of the environmental manager in the Richmond Central Office.

A5 – Problem Identification/ Background

Virginia's freshwater biological monitoring program began in the1970's to fulfill requirements of the Federal 106 grant agreement. The Virginia DEQ uses benthic macroinvertebrate communities to assess the ecological health of freshwater streams and rivers. Benthic macroinvertebrates are larger-than-microscopic invertebrate organisms such as insects, crustaceans, snails, mussels, or worms that inhabit stream bottoms.

VADEQ's biological monitoring program examines over 150 stations annually. Reasons for bioassessments include but are not limited to: targeted monitoring, probabilistic

monitoring, tracking local pollution events, follow-up on waters of concern identified through volunteer citizen monitoring, and TMDL monitoring. Data from the biological monitoring program are used in the periodic review and assessment of state waters as required by Section 305(b) of the Clean Water Act. Benthic macroinvertebrate monitoring is used in assessing the designated use of state waters established in 9 VAC 25-260-10 A. that states in part that "All state waters, including wetlands, are designated for the following uses: ……the propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might reasonably be expected to inhabit them….." .

Biological monitoring using benthic macroinvertebrates is an invaluable tool for evaluating the overall, temporally integrated effects of the water and sediment quality in streams and rivers. Benthic macroinvertebrate communities integrate water quality both through time and the effects of different pollution stressors, thus providing a holistic measure of their aggregate impact, including antagonism and/or synergism among chemical and physical pollutants. Because of their sedentary nature, macroinvertebrates are good indicators of localized conditions. Most species have a complex life cycle of approximately one year or more and therefore integrate the effects of fluctuations in water quality over time in which periodic conventional water quality surveys may miss. In essence, benthic macroinvertebrates are considered to be virtual "living recorders" of water quality conditions over time. The structure and functioning of macroinvertebrate communities are also extremely sensitive, and may exhibit responses to water quality parameters for which specific criteria or standards have not been defined, for which chemical analyses are not normally performed, or for which biological tolerance is below chemical detection limits.

The VADEQ uses two bioassessment indices to assess the biotic integrity in non-tidal freshwater streams and rivers in Virginia. In the Coastal Plain, which is characterized by low gradient streams east of the fall line, the Coastal Plain Macroinvertebrate Index (CPMI) is used. This multimetric index was developed in 1997 by the Mid-Atlantic Coastal Streams (MACS) workgroup (USEPA 1997 and Maxted et al. 2000). The CPMI is a multimetric bioassessment index which was calibrated for low gradient Coastal Plain streams which exhibit different expected benthic macroinvertebrate communities from non-coastal streams.

For non-coastal streams, biological assessment of the benthic macroinvertebrate community is based on the methods described in the EPA's Rapid Bioassessment Protocols for Streams and Rivers (RBP II) document (Plafkin et al. 1989). Unlike the CPMI which uses an established reference condition for calculation of the metrics, the RBP II requires a paired reference stream for the calculation of the metrics. The reference stream that is chosen should represent the best available or least disturbed conditions possible for the ecoregion in which the stream to be assessed is located.

In 2003, a stream condition index was developed for Virginia freshwater non-coastal streams by USEPA's contractor Tetra Tech, Inc. using historical data collected in Virginia at reference and stressed streams in 1994-1998, and was tested against additional data collected in 1999-2002. This review has resulted in the development of the Virginia Stream Condition Index (VSCI) for use in assessing wadeable non-coastal streams. It is based upon recent advances in bioassessment methods contained in "Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers, Second Edition" (Barbour et al. 1999). The VSCI, a multimetric calculation of benthic integrity converted into a single numerical score, resulted in a single reference condition for the entire non-coastal portion of the Commonwealth

against which all future benthic samples will be compared. The development of this index is considered a significant step in the advancement of the biomonitoring program to address a wide range of monitoring and assessment needs. An Academic Advisory Committee (AAC) representing a cross-section of Virginia colleges and universities, has been assembled to review the technical merits of its development, to ensure the applicability of the VSCI, and to provide recommendations toward further testing and refinement of the index.

The 2006 Integrated Report will be assessing the biological data using the same methods and metrics as have been used by VADEQ in previous 305(b) reports. DEQ intends to finalize this Stream Condition Index and use this new index to review the benthic biological data and make assessments of the biological data for the 2008 Integrated Report.

A6 – Project/ Task Description

The VADEQ Data from the biological monitoring program are used in the periodic review and assessment of state waters as required by Section 305(b) of the Clean Water Act. The following are the primary data uses:

1. 305(b) reports: Data are used to provide water quality assessments for the biennial 305(b) reports to the U.S. EPA and Congress.

2. 303(d) listing: All stream segments assessed as severely impaired and those where repeated sample data confirm moderate impairment are listed on the 303(d) list of waters prioritized for TMDL development and remediation activities.

3. Virginia Pollutant Discharge Elimination System (VPDES) permits: Some data are used in the permitting process. Biological Assessment Reports may determine if an existing discharge permit is protective of the resident fauna. If the discharge is found to impair the benthic macroinvertebrate community, the permit may be recommended to be reviewed.

4. Probabilistic monitoring: The ProbMon network is a set of randomly chosen stations used to make statistically-based assessments of Virginia's streams.

5. Tracking local pollution events: Biological data may be used to determine the effect of local pollution events in streams and to track the rate of recovery of the benthic communities in these streams.

6. Exceptional State Waters designation: Benthic macroinvertebrate data may be used to determine the exceptional aquatic communities eligibility criterion of Virginia streams and rivers to be classified as "Exceptional State Waters" (9 VAC 25-260-30 (3)).

Stream Macroinvertebrate Sampling

VADEQ uses two sampling procedures for benthic macroinvertebrates depending on stream geomorphology and instream characteristics. The single habitat sampling approach is used for streams in which riffles with appropriate substrate (cobble) are available for sampling and are large enough so that at least 2m² of the substrate can be sampled. The single habitat sampling approach is used exclusively in high gradient streams (see Appendix B i). The multihabitat sampling method is used in cases where no riffles are present, the riffles in the reach are too small and too few to sample 2m² of substrate, or the riffles in the stream are considered not the best available benthic macroinvertebrate habitat (i.e. riffles with sand or small gravel). These riffles are, however, candidates for sampling in the multi-habitat sampling if they represent at least 5% of the available substrate (see Appendix B ii). Multi-habitat sampling is most common but not limited to low gradient streams.

Habitat Assessment

Habitat assessment is conducted at each bioassessment site. Both in-stream and riparian habitat are important determinants of the composition, structure, and function of macroinvertebrate communities. Habitat quality also often is an indicator of water quality stressors in streams. In addition, poor habitat quality can obscure the effects of specific pollutants. A systematic assessment of in-stream and riparian habitat quality thus is necessary to fully assess water quality conditions in streams and rivers.

Habitat assessment is considered an important tool for the final evaluation of impairment. Habitat parameters that are evaluated are related to the overall aquatic life use and are a potential source of limitation to the aquatic biota. Both the quality and quantity of available habitat can affect the resident biological community structure and composition. The final conclusion of a bioassessment should take in consideration the habitat quality of a water body and whether the health of aquatic biological communities is limited by habitat conditions. Procedures for habitat assessments are located in Appendix B (iii).

Physicochemical Parameters

Physicochemical parameters including Dissolved Oxygen (DO), pH, conductivity, and temperature are collected at each site using several different types of multi-probe meters. These parameters may provide valuable information in determining what water physicochemical characteristics may be limiting to the health of aquatic biological communities.

Reference Site Selection

Due to the rarity of "pristine" waterways, reference sites are considered to be stream reaches that are the "least disturbed" or are considered to be in the best available condition for a certain ecoregion. Ecoregions are defined as being contiguous land forms with similar geology, soils, vegetative cover and climate and it is hypothesized that biotic communities within ecoregions are likely to be similar.

Reference sites (streams) are utilized by DEQ in the determination of non-coastal stream assessments. Test streams are assessed based on RBP II metric scores which are derived from the comparison of reference stream macroinvertebrate data to the test stream macroinvertebrate data (see Appendix C (i) for RBP II metrics and descriptions). Reference streams are chosen by regional biologist(s) based on best professional judgment in which

ecoregion boundaries, stream size, and hydrologic regime are considered so that do not differ significantly in their innate biological potential from test streams.

Streams in the Coastal Plain ecoregion are assessed using the CPMI which is based on a regional reference condition in which individual reference streams are not needed. (see Appendix C (ii) for CPMI metrics and descriptions).

A7 – Data Quality Objectives

High quality data is imperative to the DEQ's biological monitoring programs ability to accurately assess the condition of Virginia's streams and rivers. The specific data quality objectives as discussed below include accuracy and precision, representativeness, and comparability.

Accuracy and Precision

Data quality objectives for this program emphasize accuracy and precision of benthic macroinvertebrate identification at the family level of taxonomy, which will be maintained by following appropriate SOP and QA/QC procedures (Appendix C i-iv).

Representativeness

Experimental design, sampling techniques, sample preservation and sample handling are interactive factors that directly affect achievement of representativeness of benthic macroinvertebrate sampling. The experimental design for the biological monitoring program is described in section A-5 of this document. Standard Operating Procedures are utilized by the regional biologists that address station selection, sampling techniques, collection, preservation, handling, and processing to maintain standards of representativeness in the surveys.

Comparability

Comparability of biomonitoring data is a summation of quality products at each phase of the data gathering process. It includes representative sampling, sample handling procedures and procedures for reporting of biological data. Following SOPs based on published methodology, uniform sampling procedures and semi-annual training workshops ensure that regional biologists make accurate assessments of water quality statewide.

A8 – Training Requirements/ Certification

All field sampling as well as laboratory sample processing (subsorting of benthic macroinvertebrates) will be performed by or under the supervision of a professional aquatic biologist.

All taxonomic identifications will be performed by an aquatic biologist that has obtained a certification from Virginia Commonwealth University or the North American Benthological Society. Certifications are earned by passing a family level taxonomic identification proficiency test established by professional benthic macroinvertebrate taxonomists.

Agencies and organizations outside of the DEQ must submit a QAPP to the DEQ and this QAPP must be approved by biological monitoring program coordinator before their biological data will be used for assessment purposes. QAPP requirements for non-DEQ agencies and organizations are provided in the document "Guidelines for DEQ review and approval of biological monitoring QAPPs submitted by non-DEQ sources" (2006).

A9 – Documentation and Records

The QAPP for this project was written by VADEQ staff and will be sent to the appropriate EPA Region 3 QAPP contact for review. The most up-to date version of this QAPP will be available through the biological monitoring program coordinator and will also be available on DEQ's website.

All field data (habitat assessments, field observations, and water physicochemical measurements) are entered on standardized forms that are completed at the time of sampling (see Appendix D i). Water physicochemical data are later entered into CEDS in the laboratory. Lists of all identified taxa are entered and stored by station in VA EDAS, an ACCESS© database that facilitates the archiving and retrieving of taxonomic information. The EDAS database provides information that is summarized in the Agency's biennial 305(b) Water Quality Assessment Report. Results are also submitted to EPA under DEQ's Section 106 agreement.

Each regional biologist will keep originals of all field data sheets, taxonomic records, quality control records, instrument calibration records, and miscellaneous correspondence and notes related to the specific sampling stations in the appropriate dedicated storage locations. Final assessment reports will be sent the appropriate DEQ staff for each regional office.

Group B: Measurement/ Data Acquisition Elements

B1 – Sampling Process Design (Experimental Design)

The VDEQ employs two main types of sampling strategies, probabilistic monitoring and targeted monitoring. The probabilistic monitoring network is a set of randomly chosen stations used to make statistically based assessments of Virginia's streams. This approach differs from targeted monitoring by selecting stations randomly rather than biases for access or specific data needs. Data from randomly selected stations represents an unbiased distribution of statewide conditions and allows a measure of accuracy of these data.

Targeted monitoring is based on choosing stations for specific data needs such as reviewing VPDES permits, tracking local pollution events and other rationale described in section A – 6 of this document.

B2 - Sampling Methods

The sampling methods for the biological monitoring program are shown in the SOPs in Appendix B (i & ii). See section A-6 (stream macroinvertebrate sampling) for sample method determination.

B3 – Sample Handling and Custody Requirements

Each regional biologist will be responsible for the appropriate preservation, labeling, transport and storage of benthic macroinvertebrate samples. (for details see respective SOP in Appendix B). No special custody requirements of samples are required in the current program.

B4 – Analytical Methods

The SOP for benthic macroinvertebrate sub-sampling is located in Appendix B (iv).

B5 – Quality Control

Acceptable relative percent difference values and accuracy levels for quality control procedures for field and laboratory techniques for the biological monitoring program are located in Table 1.

Table 1. Quality Control Objectives for the biological monitoring program

Comparability	Accuracy and Presicion	Sorting Efficiency
The expected degree of agreement between replicate benthic macroinvertebrate samples is ≥ 70%	The expected MQO for taxonomic precision is a PTD value ≤ 10%	The expected sorting efficiency of benthic macroinvertebrate samples is ≥ 90%

Comparability- Replicate samples are taken at 10% of sampling sites. The degree of agreement is based on the percent comparability of the assessment scores between replicates. If the percent comparability is < 70%, an evaluation of the consistency of field sampling techniques may be warranted.

Accuracy and Precision- The VDEQ's Measurement Quality Objective (MQO) for taxonomic precision was suggested by the USEPA to be set at a Percent Taxonomic Disagreement (PTD) value ≤ 10%. PTD is calculated:

$$\mathrm{PTD} = \left[1 - \left(\frac{comp_{pos}}{N}\right)\right] \times 100$$

$comp_{pos}$ is the number of agreements and N is the total number of specimens in the larger of the 2 counts.

PTDs are calculated for 10% of samples taken annually from each VDEQ regional biologist and other VDEQ staff certified for taxonomic identification. Samples are sent to an EPA approved independent taxonomist for re-identification. Samples that do not meet the MQO are evaluated for the types of errors involved. Counting and transcribing errors indicate that greater attention to sample processing may need to be practiced. However, consistent MQOs greater than the suggested PTD due to taxonomic mis-identification may warrant the need for increased taxonomic identification training.

Sorting Efficiency- VDEQ staff involved in laboratory sub-sampling of samples must first demonstrate the ability to remove ≥ 90% of the specimens per grid. For detailed sub-sampling procedures and QA/QC, see Appendix B iv.

The QC officer will be responsible for conducting annual field audits to ensure appropriate SOPs are being followed in the field and lab.

B6 – Instrument / Equipment Testing, Inspecting, and Maintenance Requirements
B7 – Instrument Calibration and Frequency

Detailed information on testing, inspection, and maintenance requirements, and on calibration procedures and frequency of all multi-probe meters for measurement of stream physicochemical parameters can be found in Section IV of the "Standard Operating Procedures Manual for the Department of Environmental Quality Office of Water Quality Monitoring and Assessment" located at
www.deq.state.va.us/watermonitoring/pdf/wqmsop.pdf

B8 – Inspection/ Acceptance Requirements for Supplies and Consumables

Supplies and consumables used by the biological monitoring program are purchased through various sources. Inspections should be made before each sampling event on the D-frame dip net to ensure that there are no tears in the mesh. Sample containers should also be inspected for damage before use.

B9 –Non-direct Measurements

GIS data may be used in the determination of appropriate reference stations and to facilitate interpretation of sampling results based on watershed characteristics.

B10 – Data Management

Each regional biologist will keep originals of all field data sheets, taxonomic records, quality control records, instrument calibration records, and miscellaneous correspondence

and notes related to the specific sampling stations in the appropriate dedicated storage locations. All field data (habitat assessments, field observations, and water physicochemical measurements) are entered on standardized forms that are completed at the time of sampling (see appendix d i). Water physicochemical data are later entered into CEDS in the laboratory. Lists of all identified taxa and sub-sampling results are recorded on standardized forms (see appendix d ii) and taxa information are entered and stored by station in VA EDAS, an ACCESS© database that facilitates the archiving and retrieving of taxonomic information.

Group C: Assessment/ Oversight Elements

C1 – Assessment and Response Actions

As mentioned in section A5, the VADEQ uses two bioassessment indices to assess the biotic integrity in non-tidal freshwater streams and rivers in Virginia.

For non-coastal streams, biological assessment of the benthic macroinvertebrate community is based on the methods described in the EPA's Rapid Bioassessment Protocols for Streams and Rivers (RBP II) document (Plafkin et al. 1989). The individual metrics, metric calculations and assessment categories used for RBP II assessments are presented in Appendix C (i).

The CPMI is a multimetric bioassessment index which was calibrated for low gradient Coastal Plain streams which exhibit different expected benthic macroinvertebrate communities from non-coastal streams and developed by the MACS workgroup in 1997. The CPMI consists of five metrics: Taxonomic Richness, EPT Richness, % Dominant Taxon, Hilsenhoff Biotic Index, and Percent Clingers. The scores for each metric and assessment categories are summarized in Appendix C (ii).

For both the RBP II and CPMI indices, a bioassessment categorized as "non-impaired" results in the designation of the stream reach as "fully supporting" for Aquatic Life Use Support (ALUS). A single bioassessment categorized as "slightly impaired" or "moderately impaired" results in the designation of the stream reach as "fully supporting or insufficient data but having observed effects" where professional judgment cannot confirm impairment. If the single moderate impairment was discovered from the last 2 samples, a documented justification for not assessing as impaired is required. If the bioassessment data are confirmed to be "moderately impaired" or a single bioassessment categorized as "severely impaired", the designation of the stream reach is "impaired or threatened waters needing a TMDL. (For detailed assessment determination, see the Water Quality Assessment Guidance Manual for Y2006 located at www.deq.virginia.gov/waterguidance/pdf/052017.pdf.

Values obtained may sometimes be intermediate to established ranges and require some subjective judgment as to the assessment of biological condition. In these instances, habitat assessment, water quality data, and the Virginia Stream Condition Index (VSCI) may aid in the assessment process.

Each regional biologist is required to document any problems encountered during data collection, sample processing, or data analysis, and to take remedial action where required. Such action may include resampling or eliminating data from further consideration.

C2 – Reports to Management

Biomonitoring program staff discuss QA/QC issues at regularly scheduled meetings or as the need arises. Yearly reports will be developed by the program QC officer and distributed to the regional environmental managers and biologists. A summary of QA/QC activities , including any conditions or situations affecting data completeness or quality, corrective actions, and outcomes of corrective actions will be prepared as part of the final report.

Group D: Data Validation and Usability

D1 – Data Review, Validation, and Verification Requirements

All field and laboratory data will be reviewed, verified, and validated to ensure they conform to program specifications. It will be the responsibility of each regional environmental manager whether to accept or reject data.

D2 – Validation and Verification Methods

Data review, verification, and validation will be performed using self-assessment and peer and management review. Data will initially be validated by the regional biologist when returning from the field and further validated during entry into the EDAS database. Any errors detected will be rectified by editing incorrect database entries, resampling, or excluding questionable data. Biological data approved by the regional environmental managers will be given to the appropriate waterbody assessment personnel.

D3 – Reconciliation with Data Quality Objectives

All data collected by the biological monitoring program will be reviewed on an ongoing basis for accuracy, precision, and completeness. If data quality does not meet the appropriate specifications, data will be discarded and resampling may occur.

References

Barbour, M.T., J. Gerritsen, and B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers; periphyton, benthic macroinvertebrates, and fish 2nd edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-b-99-002

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Appendix A

List of Acronyms

AAC	Academic Advisory Committee
ALUS	Aquatic Life Use Support
CEDS	Comprehensive Environmental Data System
CO	Central Office
CPMI	Coastal Plain Macroinvertebrate Index
EDAS	Ecological Data Application System
GIS	Geographical Information Systems
MACS	Mid-Atlantic Coastal Streams
MQO	Measurement Quality Objective
NRO	Northern Regional Office
PTD	Percent Taxonomic Agreement
PRO	Piedmont Regional Office
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RBP II	Rapid Bioassessment Protocols (II)
SCRO	South Central Regional Office
SOP	Standard Operating Procedure
SWRO	South West Regional Office
TMDL	Total Maximum Daily Load
TRO	Tidewater Regional Office
USEPA	United States Environmental Protection Agency
VADEQ	Virginia Department of Environmental Quality
VPDES	Virginia Pollutant Discharge Elimination System
VRO	Valley Regional Office
VSCI	Virginia Stream Condition Index
WCRO	West Central Regional Office

Appendix B (i)

SOP Title: Methods for Benthic Macroinvertebrate Collections in Cobble Substrate (single habitat)

Date of Last Revision: 2/22/2006

Equipment/Materials: standard aquatic dip net, D-frame (500-µm mesh openings), 0.3 meter width (~1 foot), sieve bucket, (500-µm mesh openings), wash bucket, 70 percent isopropyl, sample containers, forceps, field notebook, pencils, first aid kit

References:

Barbour, M.T., J. Gerritsen, and B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers; periphyton, benthic macroinvertebrates, and fish 2nd edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-b-99-002

Procedures:

Habitat:	riffles, runs
Area:	2m² total; 6 kicks of 1 meter or 12 kicks of ½ meter
Mesh Size	500-µm mesh openings
Index Period	regional consideration or sample reference sites during same period; decisions based on project/program objectives

1. The sample reach (considered to be a station) should extend to a 100-meter instream segment of habitat having no major tributaries in the assessment area. Sampling should be conducted at least 100-meters upstream of any road or bridge crossing to minimize the affects on stream velocity, depth and overall habitat

2. Starting at the downstream end of the reach and moving upstream, all riffles and runs are candidates for sampling throughout the reach. Sampling is conducted holding the dipnet on the bottom of the stream and kicking the cobble substrate (i.e., riffles and runs) to agitate and dislodge organisms. A single kick consists of disturbing the substrate upstream of the net by kicking with the feet and/or by using the hands to dislodge the cobble. Six kicks one meter above the dipnet or 12 kicks of half a meter above dipnet should be used to sample a total of 2m².

3. *Riffles/Runs* – Shallow part of the stream where water flows swiftly over completely or partially submerged pebble to boulder sized rocks to produce surface agitation. Sample by holding the bottom rim of the dip net against the substrate downstream of the riffle and perpendicular to the flow while disturbing the substrate just upstream of the net with feet and hands to dislodge organisms.

4. The collected sample is washed by running clean stream water through the net 2-3 times; transfer the sample to the sieve bucket. Do not let the net become so clogged with debris that it results in the diversion of water around the net rather than through the net. If

clogging occurs, discard the sample in the net and redo that portion of the sample in a different location.

5. As the sample is added to the sieve bucket, it should be further washed to remove fines. Mix the sample by hand while sieving, remove large debris from the sample after rinsing and inspecting for organisms; place any organisms back into the sieve bucket. Do not attempt to inspect small debris.

6. Transfer the sample from the sieve bucket to prelabeled sample container(s) and preserve in 70 percent isopropyl alcohol. Forceps may be needed to remove organisms from the screen and dipnet.

7. Complete the Benthic Macroinvertebrate Field Data Sheet including comments on weather and wildlife observations etc. Notes on the stable habitats sampled should be recorded. (i.e., the proportion of snags, vegetation, etc. sampled; type of substrate; condition of habitats).

Quality Control (QC)

8. Field sampling QC involves the collection of replicate samples at various reaches to verify the repeatability of the results obtained by a single set of field investigators. Each investigation team should conduct replicate sampling at 10 percent of the sampling reaches. Replicate sampling is conducted on an adjacent reach upstream of the initial sampling. The adjacent reach should be similar to the initial site in respect to habitat, stressors, point source pollution, etc. Replicate samples are preserved, subsampled, and organisms are identified using SOPs. Results are recorded in a sampling QC log book.

9. Sample Labels forms should include the following information: station ID, date and time, preservative, habitat sampled, and sampler's name.

Appendix B (ii).

SOP Title: Methods for Multi-habitat Benthic Macroinvertebrate Collections

Date of Last Revision: 2/22/2006

Equipment/Materials: standard aquatic dip net, D-frame (500-µm mesh openings), 0.3 meter width (~1 foot), sieve bucket, (500-µm mesh openings), wash bucket, 70 percent isopropyl, sample containers, forceps, field notebook, pencils and first aid kit

References:

United States Environmental Protection Agency. 1997. Field and laboratory methods for macroinvertebrate and habitat assessment of low-gradient nontidal streams. Mid-Atlantic Coastal Streams Workgroup, Environmental Services Division, Region 3, Wheeling, W.V

Barbour, M.T., J. Gerritsen, and B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers; periphyton, benthic macroinvertebrates, and fish 2nd edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-b-99-002

Procedures:

Habitat:	snags, vegetation, banks, riffles
Area:	20 jabs, each 1-m in length
Mesh size:	500-µm mesh openings
Index Period	regional consideration or sample reference sites during same period; decisions based on project/program objectives

1. The sample reach (considered to be a station) should extend to a 100-meter instream segment of habitat having no major tributaries in the assessment area. Sampling should be conducted at least 100-meters upstream of any road or bridge crossing to minimize the affects on stream velocity, depth and overall habitat.

2. Sampling is conducted from downstream to upstream by jabbing the D-frame net into productive and stable habitats 20 times. A single jab consists of forcefully thrusting the net into a productive habitat for a linear distance of 1-meter, followed by 2-3 sweeps of the same area to collect dislodged organisms.

3. Different types of habitat should be sampled in rough proportion to their frequency within the reach. Unique habitat types (i.e., those consisting of less than 5 percent of stable habitat within the sampling reach) should not be sampled.

4. Identify proportional representation of habitat types. Characterize the bottom and shore-zones according to features present at the time the example is collected. Do not base characterizations on anticipated oscillations of flow regime or substrate compositions.

    a) Bottom-zone (within channel substrate)

- Riffles have relatively fast velocity, shallow steam depth, steep surface gradient and a straight to convex channel profile. Riffles are usually topographic high areas produced by the accumulation of coarse materials.
- Non-riffle encompasses all other forms (e.g., pools runs, and slack areas) and generally possess intermediated to fine particle substrate.
- Vegetation such as submerged macrophytes serve as habitat for macroinvertebrates and may constitute large areas of the available substrate.

b) Shore-zone (allochthonous material)
- Overhanging vegetation includes terrestrial shore-zone plant material that is living, submerged, and provides in-stream cover for fish and macroinvertebrates.
- Submerged tree roots include living root material from shoreline or overhanging vegetation that is submerged and provides in-stream cover for fish macroinvertebrates.
- Woody debris includes submerged snags and/or other woody material microbial conditioning. Woody debris in channel is considered part of shoreline for estimating allocation of sampling.

5. Proportionally allocate sampling effort (20 jabs/sweeps/kicks) to shore-zone and bottom-zone.

6. The collected sample is washed by running clean stream water through the net 2-3 times; transfer the sample to the sieve bucket. Samples should be cleaned and transferred to the sieve bucket at least every five jabs, more often if necessary. Do not let the net become so clogged with debris that it results in the diversion of water around the net rather than through the net. If clogging occurs, discard the sample in the net and redo that portion of the sample in a different location.

7. As the sample is added to the sieve bucket, it should be further washed to remove fines. Mix the sample by hand while sieving, remove large debris from the sample after rinsing and inspecting of organisms; place any organisms back into the sieve bucket. Do not attempt to inspect small debris.

8. Transfer the sample from the sieve bucket to pre-labeled sample container(s) and preserve in 70 percent isopropyl alcohol. Forceps may be needed to remove organisms from the sieve screen and dipnet.

Following are specific sampling techniques for different productive and stable habitats:

Riffles/Runs – Shallow part of the stream where water flows swiftly over completely or partially submerged pebble to boulder sized rocks to produce surface agitation. Sample by holding the bottom rim of the dip net against the substrate downstream of the riffle and perpendicular to the flow while disturbing the substrate just upstream of the net with feet and hands to dislodge organisms.

Snags- Submerged woody debris, sampled by jabbing in medium-sized snag material (sticks and branches). The 1-meter section of this habitat is estimated. The snag habitat may be kicked first to help dislodge organisms, but do so only after placing net in water downstream of the snag. Accumulated woody material in pool areas can also be considered as snag habitat.

Vegetation – Aquatic plants that are rooted on the bottom of the stream; they are sampled in deep water by drawing the net through the vegetation from the bottom to the surface of the water; in shallow water they are sampled by bumping the net along the bottom in the rooted area.

Banks – When banks have roots, plants, and snags associated with them, they are sampled in a fashion similar to snags. When the banks are of unvegetated or soft soil, they are sampled by bumping the net along substrate rather than dragging the net through soft substrates; this will reduce the amount of detritus (defined as sticks, leaves, and/or pieces of bark) through which you would have to pick. Also, the bank habitat can be kicked first in order to help dislodge organisms.

9. Complete the Benthic Macroinvertebrate Field Data Sheet including comments on weather and wildlife observations etc. Notes on the stable habitats sampled should be recorded. (i.e., the proportion of snags, vegetation, etc. sampled; type of substrate; condition of habitats). Also note how the samples were collected; if wading in-stream, walking on the banks, out of channel, from a boat, etc.

Quality Control (QC)

10. Field sampling QC involves the collection of replicate samples at various reaches to verify the repeatability of the results obtained by a single set of field investigators. Each investigation team should conduct replicate sampling at 10 percent of the sampling reaches. Replicate sampling is conducted on an adjacent reach upstream of the initial sampling. The adjacent reach should be similar to the initial site in respect to habitat, stressors, point source pollution, etc. Replicate samples are preserved, sub-sampled, and organisms are identified using SOPs. Results are recorded in a sampling QC log book.

11. Sample Labels should include the following information; station ID, date and time, preservative, habitat sampled, and sampler's name.

Appendix B (iii)

SOP Title: Methods for Habitat Assessment for Streams

Date of Last Revision: 2/22/2006

Equipment/Materials: Habitat Assessment Field Sheets for (1) High Gradient Streams and (2) Low Gradient Streams, pencils, field notebook

References:
Barbour, M.T., J. Gerritsen, and B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers; periphyton, benthic macroinvertebrates, and fish 2nd edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-b-99-002

Procedures:

1. Select the reaches for conducting the habitat assessment and complete sections on general characteristics and land use.

2. The habitat assessment will be focused on evaluating the physical habitat structure of a 100-meter section of the stream and upper reaches in the catchment for the large-scale parameters.

    a) Identify the lowest most point of the stream that was sampled for macroinvertebrates. Measure a 100-meter section that is consistent with the biological sampling reach to assess large-scale parameters.

    b) Complete the identifying information on the field data sheets for the habitat assessment.

Physical Habitat Structure:

3. Conduct the habitat assessment. Refer to the descriptors described here and the decision criteria on the habitat assessment field data sheet.

The following information describes the parameters that will be evaluated for high-gradient stream habitats. The first 5 parameters are assessed directly in the entire 100-meter reach that was used for the macroinvertebrate sampling.

4. Epifaunal substrate/available cover (parameter #1 includes the relative quantity and variety of natural structures in the stream, such as fallen trees, logs, and branches cobble and large rocks, and undercut banks that are available to fish and macroinvertebrates for refugia, spawning/nursery activities, or feeding. A wide variety of submerged structures in the stream provide aquatic organisms with many living spaces; the more living spaces in a stream, the more types of organisms the stream can support.

5. Embeddedness (Parameter #2) refers to the extent to which rocks (gravel, cobble and boulders) are surrounded by, covered or sunken into the silt, sand, or mud of the stream

bottom. Generally, as rocks become embedded, fewer living spaces are available to macroinvertebrates and fish for shelter, spawning and egg incubation. This parameter is assessed primarily in the riffles, if present. To estimate the percent of embeddedness, observe the amount of silt or finer sediments surrounding the rocks. If kicking does not dislodge the rocks or cobbles, they may be greatly embedded. It may be useful to lift a few rocks and observe how much of the rock (e.g., ½, ⅓) is darker due to anoxic reaction to the inorganic surface.

6. Velocity/Depth regime is important to the maintenance of healthy aquatic communities. Fast water increases the amount of dissolved oxygen in the water, keeps pools from being filled with sediment, and helps food items like leaves, twigs, and algae move more quickly through the aquatic system. Slow water provides spawning areas for fish and shelters macroinvertebrates that might be washed downstream in higher stream velocities. Similarly, shallow water tends to be more easily aerated (i.e., hold more oxygen), but deeper water stays cooler longer. Thus the best stream habitat will include all of the following velocity/depth combinations, and can maintain a wide variety of organisms.

    a) slow (<0.3 m/sec), shallow (<0.5 m)
    b) slow, deep
    c) fast, deep
    d) fast, shallow

7. Sediment deposition is a measure of the amount of sediment that has been deposited in the stream channel and the changes to the stream bottom that have occurred as a result of the deposition. Excessive levels of sediment deposition create an unstable and continually changing environment that is unsuitable for many aquatic organisms. Sediments are naturally deposited in areas where flow is obstructed. These deposits can lead to the formation of islands, shoals, or point bars (sediments that build up in the stream, usually at the beginning of a meander) or can results in the complete filling of pools. To determine whether or not these sediment deposits are new, look for vegetation growing on them: new sediments will not yet have been colonized by vegetation.

8. Channel flow status determines the percent of the channel that is filled with water. The flow status will change as the channel enlarges or as flow decreases as a result of dams and other obstructions, diversions for irrigation, or drought. When water does not cover much of the streambed, less living area is available for aquatic organisms. Assess the wetted width of the stream in relation to the location of the lower bank.

    The next 5 parameters should be assessed along a length of stream that includes the sampling reach plus 1 or 2 reaches upstream.

9. Channel alteration is basically a measure of large-scale changes in the shape of the stream channel. Many streams in urban and agricultural areas have been straightened, deepened (e.g. dredged), or diverted into concrete channesl, often for flood control purposes. Such streams have far fewer natural habitats for fish, macroinvertebrates, and plants than do naturally meandering streams. Channel alteration is present when the stream runs through a concrete channel; when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when combined sewer overflows

(CSOs) pipes are present; when the stream is of uniform depth due to dredging; and when other such changes have occurred. Signs that indicate the occurrence of dredging include straightened, deepened, and otherwise equipment access to the stream.

10. Frequency of riffles (or bends) is a way to measure the heterogeneity occurring in a stream. Because riffles are a good source of high-quality habitat and faunal diversity, an increase in the frequency of riffles provides for greater diversity of the stream community. In streams where riffles are uncommon, a measure of the frequency of bends can be used as a measure of meandering or sinuosity, which also provides for a diverse habitat and fauna. Additionally, streams with a high degree of sinuosity are better suited to handle storm surges through absorption of energy by bends as well as providing refugia for fauna during storm events.

For the last 3 parameters, visually evaluate the condition of the right and left stream banks, separately. Face downstream to determine left from right. Assess these parameters along the stream margins for the sampling reach as well as 1 or 2 adjacent reaches.

11. Bank stability measures erosion potential and whether the stream banks are eroded. Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks and are therefore considered to have high erosion potential. Signs of erosion include crumbling, unvegetated banks, exposed tree roots, and exposed soil.

12. Bank vegetative protection measures the amount of the stream bank that is covered by natural (i.e., growing wild and not obviously planted) vegetation. The root systems of plants growing on stream banks help hold soil in place, reducing erosion. Vegetation on banks provides shade for fish and macroinvertebrates, and serves as a food source by dropping leaves and other organic matter into the stream. Ideally, a variety of vegetation should be present, including trees, shrubs, and grasses. Vegetative disruption may occur when the grasses and plants on the streambanks are mowed or grazed upon, or the trees and shrubs are cut back or cleared.

13. The riparian vegetative zone width is defined here as the width of natural vegetation from the edge of the stream bank. The riparian vegetative zone is a buffer zone to pollutants entering a stream from runoff; it also controls erosion and provides stream habitat and nutrient input into the stream. A wide, relatively undisturbed riparian vegetative zone reflects a healthy stream system; narrow, far less useful riparian zones occur when roads, parking lots, fields, lawns and other artificially cultivated areas, bare soil, rocks, or buildings are near the stream bank. The presence of "old fields" (i.e., previously developed agricultural fields allowed to convert to natural conditions) should rate higher than fields in continuous or periodic use.

The following information describes the parameters that will be evaluated for low-gradient stream habitats.

14. Epifaunal substrate/available cover includes the relative quantity and variety of natural structures in the stream, such as fallen trees, logs, and branches, cobble and large rocks, and undercut banks, that are available to fish and macroinvertebrates for refugia, spawning/nursery activities, or feeding. A wide variety of submerged structures in the

stream provide aquatic organisms with many living spaces; the more living spaces in a stream, the more types of organisms the stream can support.

15. Pool substrate characterization refers to the type and condition of bottom substrates found in pools Firmer sediment types (e.g., gravel, sand) and rooted aquatic plants support a wider array of organisms that pools dominated by mud or bedrock and with little or no plants. Additionally, streams with a variety of substrate types will support far more types of organisms than streams with uniform pool substrates.

16. Pool variability rates the overall mixture of pool types found in streams according to size and depth. Streams with many pool types support a wider variety of organisms than streams with fewer pool types. Thus the best stream habitat will include all of the following pool types, and can maintain a wider variety of aquatic species.

    a) large (>half cross-section of stream) –shallow (<1.0 m)
    b) large-deep
    c) small-shallow
    d) small-deep

17. Sediment deposition is a measure of the amount of sediment that has been deposited in the stream channel and the changes to the stream bottom that have occurred as a result of the deposition. Excessive levels of sediment deposition create an unstable and continually changing environment that is unsuitable for many aquatic organisms. Sediments are naturally deposited in areas where the stream flow is reduced, such as pools and bends, or where flow is obstructed. These deposits can lead to the formation of islands, shoals, or point bars (sediments that build up in the stream, usually at the beginning of a meander) or can result in the complete filling of pools. To determine whether or not these sediment deposits are new, look for vegetation growing on them: new sediments will not yet have been colonized by vegetation.

18. Channel flow status determines the percent of the channel that is filled with water. The flow status will change as the channel enlarges or as flow decreases as a result of dams and other obstructions, diversions for irrigation, or drought. When water does not cover much of the streambed, less living area is available for aquatic organisms. Assess the wetted width of the stream in relation to the location of the lower bank.

The next 5 parameters should be assessed along a length of stream that includes the sampling reach plus one or two reaches upstream.

19. Channel alteration is basically a measure of large-scale changes in the shape of the stream channel. Many streams in urban and agricultural areas have been straightened, deepened (e.g., dredged), or diverted into concrete channels, often for flood control purposes. Such streams have far fewer natural habitats for fish, macroinvertebrates, and plants that do naturally meandering streams. Channel alteration is present when the stream runs through a concrete channel; when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when the stream is very straight for significant distances; when dams, bridges and flow-altering structures, such as combined

sewer overflow (CSOs) pipes are present; when the stream is of uniform depth due to dredging; and when other such changes have occurred. Signs that indicate the occurrence of dredging include straightened, deepened, and otherwise uniform stream channels, and the removal of streamside vegetation to provide dredging equipment access to the stream.

20. Channel sinuosity is a way to measure the meandering or sinuosity occurring in a stream. A stream with a high degree sinuosity provides for a more diverse habitat and fauna than a stream with a low degree of sinuosity. Additionally; streams with a high degree of sinuosity are better suited to handle storm surges through absorption of energy by bends as well as providing refugia for fauna during storm events.

For the last 3 parameters, visually evaluate the condition of the right and left stream banks, separately. Face downstream to determine left from right. Assess these parameters along the stream margins for the sampling reach as well as 1 or 2 adjacent reaches.

21. Bank stability measures erosion potential and whether the stream banks are eroded. Steep banks are more likely to collapse and suffer from erosion that are gently sloping banks and are therefore considered to have a high erosion potential. Signs of erosion include crumbling, unvegetated banks, exposed tree roots, and exposed soil.

22. Bank vegetative protection measures the amount of the stream bank that is covered by natural vegetation (i.e., growing on stream banks) which helps hold soil in place, reducing erosion. Vegetation on banks provides shade for fish and macroinvertebrates, and serves as a food source by dropping leaves and other organic matter into the stream. Ideally, a variety of vegetation should be present, including trees, shrubs , and grasses. Vegetative disruption may occur when the grasses and plants on the streambanks are mowed or grazed upon, or the trees and shrubs are out back or cleared.

23. The riparian vegetative zone width is defined here as the width of natural vegetation from the edge of the stream bank. The riparian vegetative zone is a buffer zone to pollutants entering a stream from runoff; it also controls erosion and provides stream habitat and nutrient input into the stream. A wide, relatively undisturbed riparian vegetative zone reflects a healthy stream system; narrow, far les useful riparian zones occur when roads, parking lots, fields, lawns and other artificially cultivated areas, bare soil, rocks, or buildings are near the stream bank. The presence of “old fields” (i.e., previously developed agricultural fields allowed to convert to natural conditions) should rate higher than fields in continuous or periodic use.

To perform the habitat assessment data analysis, rate each of the 10 parameters and combine the ratings into a single index score.

24. Perform habitat assessment data analysis. To properly the evaluate condition of the stream site, compare it to an optimal or best condition found in the region (reference condition). In an ideal world, the reference condition would reflect the water quality, habitat, and aquatic life characteristics of pristine sites in the same ecological region as your stream. In real life, however, few pristine sites remain. The reference condition is therefore generally a composite of sites that reflect the best physical, chemical, and biological conditions existing in the ecological region. To make this comparison to reference conditions, divide the index score for your stream site by the score for the

reference conditions and multiply by 100 to obtain a percent similarity. Compare the result to the table below to obtain the habitat quality category for your site.

Reference Scores for Sampling Site Comparison

Percent Similarly to Reference Score[a]	Habitat Quality Category	Attributes
≥90%	Excellent	Comparable to the best situation to be expected within an ecoregion. Execellent overall habitat structure conducive to supporting healthy biological community/
75-88%	Good	Habitat structure slightly impaired. Generally, diverse instream habitat well –developed; some degradation of riparian zone and banks; a small amount of channel alteration may be present
60 – 73%	Fair	Loss of habitat compared to reference. Habitat is a major limiting factor to supporting a health biological community
≤ 58%	Poor	Severe habitat alteration at all levels.

[a]If your score falls at or near the break between habitat quality categories, use your best judgment to determine appropriate rating.

25. Perform quality control on datasheets. Habitat assessment sheets and any field data sheets should be filled out as accurately and completely as possible. All field data sheets should be properly labeled and filled.

Habitat assessments are subjective evaluations and are potentially subject to variability among investigators. Minimize variability by proper training, discuss habitat parameters and conduct evaluations as a team. See Barbour et al. (1999) for more specific guidance.

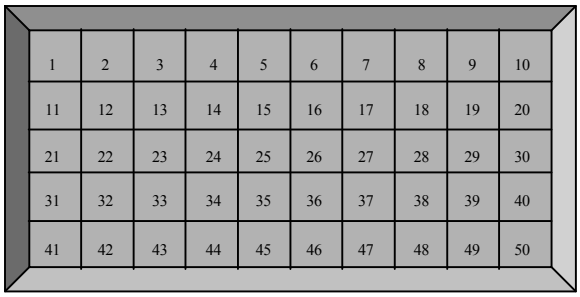
Appendix B (iv)

Title: Methods for Laboratory Sorting and Subsampling of Benthic Macroinvertebrate Samples

Date of Last Revision: 12/20/2005

Equipment/Materials:

Forceps
Standardized gridded tray (500 µm Screen, 50 quadrants, each 25 cm^2)
Scissors
Small putty knife
Quadrant-sized square metal "cookie cutter"
White plastic or enamel pan for sorting
70% isopropyl
Specimen vials, caps or stoppers
Sample labels
Dissecting microscope for organism identification (10-40x)
Macroinvertebrate Log Book
Benthic Macroinvertebrate Subsampling bench sheet



Subsampling Tray

References:

Caton, L. W. 1991. Improved sub-sampling methods for the EPA "Rapid Bioassessment" Benthic protocols. Bulletin for the North American Benthological Society 8(3):317-319.

Barbour, M.T., J. Gerritsen, and B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers: periphyton, benthic macroinvertebrates, and fish, 2nd Edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-B-00-002.

General:

The sorting and subsampling of the macroinvertebrate samples in the laboratory facilitates processing and identification of organisms collected in wadeable streams. Sample receipt is recorded at the laboratory in the Macroinvertebrate Log. A randomized 100-organism sub-sample is sorted and preserved using a special Caton gridded tray and screen, designed by Larry Caton, Oregon Department of Environmental Quality (Caton, 1991). Documentation for the level of effort, or proportion of sample processed, is recorded on the Benthic Macroinvertebrate Laboratory Bench Sheet.

Internal Label Information Required for each Vial of Sorted Material:

- Station Name
- Station Location
- Station ID
- Sorter's Initials
- "1 of 2" "2 of 2" if necessary

Procedures:

1. Log each sample (as it is received) on the Benthic Sample Log-in sheet. Store the samples at room temperature until ready for processing.

2. Remove the lid from the sample container and pull out the internal sample label (save the sample label – it will need to be returned to the sample container with the archived portion of the sample that does not get processed). Record sample collection information on the Benthic Macroinvertebrate Laboratory Bench Sheet. Header information required includes station ID, station location, station number, sample type, date the sample was collected, and the field team who collected the sample. Also, record the sorting date each sample was completed near the top right corner of the bench sheet and also in the appropriate place on the Benthic Sample Log-In Sheet.

3. Enter the sorter's initials in the appropriate column on the bench sheet for each grid that is sorted.

4. Transfer the homogenized sample material to the gridded Caton tray (use more than one sub-sampling device if necessary). Wash the sample thoroughly by running tap water over it to remove any fine material.

5. Place the gridded tray into a larger tray or sink. Add enough water to spread the sample evenly throughout the Caton grid (the water level should be relatively close to the top of the tray). Spread the sample material over the bottom of the pan as evenly as possible. Move the sample into the corners of the pan using forceps, spoon, or by hand. Vibrate or shake the pan gently to help spread the sample.

6. Lift the screen out of the larger tray or sink to drain.

7. Use a random number generator to select a grid to process. Remove all the material from that grid and place the removed material into a separate holding container, such as a white plastic or enamel pan. The material is removed as follows:

    a. Place the metal dividing frame or "cookie cutter" over the sample at the approximate location of the grid selected for processing (based on the numbers marked on the sides of the gridded tray). Use a pair of rulers or other straight edges to facilitate lining up the cookie cutter at the intersection if necessary.

b. Removed the material within the "cookie cutter" using the putty knife, a teaspoon, or forceps. Depending on the consistency of what is in the sample, it might be necessary to cut the material along the outside of the "cookie cutter" with scissors or putty knife so that only one grid's worth of sample material is used. Inspect the screen for any remaining organisms. Use the following rules when dealing with organisms that lie on the line between two grids:

- An organism belongs to the grid containing its head.

- If it is not possible to determine the location of the head (i.e., for worms), the organism is considered to be in the grid containing most of its body.

- If the head of an organism lies on the line between two grids, all organisms on the top of a grid and those on the right side of a grid belong in that grid, and are picked with that grid

c. Place the material from the selected grid(s) into a separate white plastic or enamel pan. Add the necessary amount of water to the pan to facilitate sorting.

8. Completely remove all macroinvertebrates from the selected quadrate and store organisms in a vial of 70% alcohol. If more than 40 organisms are selected from the first quadrate, skip to step 9.

a. Remove the macroinvertebrates from the detritus by using forceps or by transferring a spoonful of the material to a Petri dish for examination beneath a dissecting microscope; place picked organisms in an internally-labeled vial (or larger container, if necessary) containing 70% isopropyl as a preservative. Keep a count of the number of organisms removed and enter the number or organisms found in each grid under that column on the Benthic Macroinvertebrate Laboratory Bench Sheet.

b. Continue selecting and processing randomly selected quadrates until 100 organisms are counted. **Each grid begun must be picked to completion; that is, even if the target is reached halfway through a grid, finish the entire grid. A minimum of 4 grids must be picked.**

c. Do not remove or count empty snail or bivalve shells, pupae, or incidentally-collected terrestrial taxa. Also do not count fragments such as legs, antennae, gills, or wings. For Oligochaeta, attempt to remove and count only whole organisms and fragments that include the head; do not count fragments that do not include the head.

d. If the last grid being processed results in more than 120 organisms (i.e., 20% above target number), evenly redistribute all of the organisms (without detritus) in a gridded dish or tray containing water to cover.

Use a random numbers table and remove organisms to obtain subsample numbering between 100 and 120 organisms. The remaining organisms in the tray may be discarded.

e. Record the number of quadrates in the subsample (use multipliers from table for high density samples).

f. Identify all organisms in the sample to family, record on the benthic Macroinvertebrate Bench Sheet, and enter data into EDAS

9. Processing of high density samples

a. Discard all of the organisms picked from the first quadrate

b. Using a random numbers table, take the number of quadrates designated by the table below **all at once** depending on the number of organisms in the first quadrate. Place the selected quadrates in the sorting tray, then discard the remaining sample and clean out the subsampling box.

c. If the first quadrate had more than 400 organisms, this process will have to be repeated again.

d. Completely mix the selected quadrates in the tray and go back to step 8 a-f.

Organisms per quadrate in original sample	Remove following number of quadrates	Predicted number of organisms per quadrate	Predicted number of quadrates to reach 100	Multiplier for recording total number of quadrates picked
40-75	20	16-30	4-7	0.4
76-100	15	22.8-30	4-7	0.3
101-150	10	20.2-30	5-7	0.2
151-400	5	15.1-40	3*-7	0.1

*4 quadrates will be removed anyway, though it may lead to a subsample of up to 160 organisms. In this case, subsampling will continue as described in step 8d.

Documentation:

1. Log samples on the Bench Log-In Sheet as they are received in the laboratory.

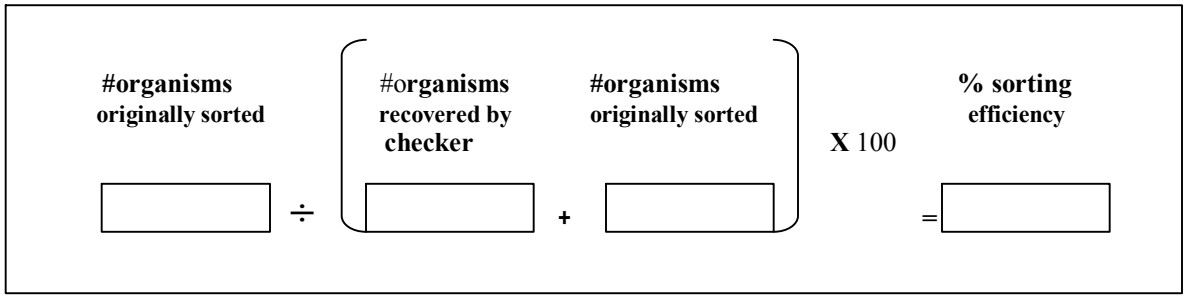
2. Complete a Benthic Macroinvertebrate Laboratory Bench Sheet for each sample as it is processed.

Quality Assurance/Quality Control

Because it can be difficult to detect the organisms in stream samples (due to inexperience, detritus, etc.), only persons who have received instruction by senior biology staff familiar with processing benthic samples can perform a quality control (QC) check.. These QC checks must be performed immediately following sorting of each grid. Therefore, a laboratory staff member qualified to perform QC checks must be present anytime samples are processed by an inexperienced individual.

1. Initially, experienced personnel will check all sorted grids from the first five samples processed by a sorter to ensure that all organisms were removed from the detritus. This will not only apply to inexperienced sorters, but also to those initially deemed as "experienced." Qualification will only occur when sorters are consistent in achieving ≥ 90% sorting efficiency after at least five samples have been checked.

2. The QC checker will calculate sorting efficiency for each sample (number of organisms/sample found by the initial sorter ÷ total number of organisms/sample found by QC Officers × 100 = %), If sorting efficiency for each of these five consecutive samples is ≥ 90% for a particular individual, this individual is considered "experienced" and can serve as a QC checker. In the event that an individual fails to achieve ≥ 90% sorting efficiency, they will be required to sort an additional five samples in order to continue to monitor their sorting efficiency. However, if they show marked improvement in their sorting efficiency prior to completion of the next five samples, whereby they acquire the ≥ 90% sorting efficiency, the QC checker may, at his/her discretion, consider this individual to be "experienced." Sorting efficiency should not be calculated for samples processed by more than one individual.



Appendix C (i)

Rapid Bioassessment Protocols (RBP II): Metric scoring criteria, assessment categories, and metric definitions

Metric	Metric Scoring Criteria			
	6	4	2	0
1. Taxa Richness (a)	>80%	60-80%	40-60%	<40%
2. Family Biotic Index (modified) (b)	>85%	70-85%	50-70%	<50%
3. Ratio of Scrapers/Filt. Collectors (a,c)	>50%	35-50%	20-35%	<20%
4. Ratio of EPT and Chironomid Abundances (a)	>75%	50-75%	25-50%	<25%
5. % Contribution of Dominant Family (d)	<20%	20-30%	30-40%	>40%
6. EPT Index (a)	>90%	80-90%	70-80%	<70%
7. Community Loss Index (e)	<0.5	0.5-1.5	1.5-4.0	>4.0
8. Ratio of Shredders/Total (a,c)	>50%	35-50%	20-35%	<20%

(a) Score is a ratio of study site to reference site x 100.

(b) Score is a ratio of reference site to study site x 100.

(c) Determination of Fuctional Feeding Group is independent of taxonomic grouping.

(d) Scoring criteria evaluate actual percent contribution, not percent comparability to the reference station.

(e) Range of values obtained. A comparison to the reference station in incorporated into these indices.

Total Possible Score = 48

Assessment Category	Score Range
Non-impaired	40 - 48
Slightly Impaired	26 - 38
Moderately Impaired	10 - 24
Severely Impaired	0 - 8

Metric	Definition	Response to increased pertubation
1. Taxa Richness (Total taxa)	Measures the overall variety of the macroinvertebrate assemblage	Decrease
2. Family Biotic Index (modified)	Uses tolerance values to weight abundance in an estimate of overall pollution	Increase
3. Ratio of Scrapers/ Filt. Collectors	Reflects the riffle/run food base.	Decrease
4. Ratio of EPT and Chironomid Abundances	Uses the abundance ratio of Chironomidae to the more sensive insect groups	Decrease
5. % Contribution of Dominant Family	Uses abundance of the numerically dominant taxon relative to the rest of the population as an indication of community balance.	Increase
6. EPT Index	Number of taxa in the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Tricoptera (caddisflies)	Decrease
7. Community Loss Index	Measures the loss of benthic taxa between a reference station and the station of comparison	Increase
8. Ratio of Shredders/Total	Allows evaluation of potential impairment as indicated by the Shredder community	Decrease

Appendix C (ii)

The Coastal Plain Macroinvertebrate Index (CPMI): Metric scoring criteria, assessment categories, and metric definitions

	Metric Scoring Criteria			
Metric	6	4	2	0
1. Total Taxa	>17	12-17	6-11	<6
2. EPT Taxa	>6	5-6	3-4	<3
3. % Ephemeroptera	>24%	16-24%	8-15%	<8%
4. HBI	<5.7	5.7-6.4	6.5-7.2	>7.2
5. % Clingers	>26%	18-26%	9-17%	<9%

Total Possible Score = 30

Assessment Category	**Score Range**
Non-impaired	24 - 30
Slightly Impaired	16 - 22
Moderately Impaired	6 - 14
Severely Impaired	0 - 4

Metric	**Definition**	**Response to increased pertubation**
Total Taxa	Measures the overall variety of the macroinvertebrate assemblage	Decrease
EPT Taxa	Number of taxa in the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Tricoptera (caddisflies)	Decrease
% Ephemeroptera	Percent of mayfly nymphs	Decrease
Hilsenoff Biotic Index (HBI)	Uses tolerance values to weight abundance in an estimate of overall pollution	Increase
% Clingers	Percent of insects having fixed retreats or adaptations for attachment to surfaces in flowing water	Decrease

Appendix D (i)

Benthic Macroinvertebrate Field Data Sheet (front)

Station ID: _____ Ecoregion _____

Field Team: _____ Survey Reason: _____

Stream Name: _____ Land Use: _____

Location: _____

DATE _____ Start Time _____ Finish Time _____

LATITUDE (Decimal degrees) _____ LONGITUDE (Decimal degrees) _____ GPS Signal _____

Stream Physicochemical Measurements

Instrument ID number:_____

TEMPERATURE:_____ºC CONDUCTIVITY:_____µS/cm

DISSOLVED OXYGEN:_____mg/L pH: _____

Did instrument pass all post-calibration checks?:_____
(If NO- which parameter(s) failed and action taken: _____

Benthic Macroinvertebrate Collection

Method used (circle one) **Single habitat** **Multi-habitat**

Riffle quality (circle one) **Good** **Marginal** **Poor** **None**

Habitats sampled	**Riffle**	**Snags**	**Banks**	**Vegetation**
# jabs	____	____	____	_____

Perservative:_____ Area Sampled (square meters) :_____

RBP Habitat Assessment

	High Gradient		*Low Gradient*	
1	Epifaunal Substrate		Epifaunal Substrate	
2	Embeddedness		Pool Substrate	
3	Velocity/Depth		Pool Variability	
4	Sediment Deposition		Sediment Deposition	
5	Channel Flow Status		Channel Flow Status	
6	Channel Alteration		Channel Alteration	
7	Riffle Frequency		Channel Sinuosity	
8	Bank Stability LDB 1-10		Bank Stability LDB 1-10	
	Bank Stability RDB 1-10		Bank Stability RDB 1-10	
9	Vegetative Protection LDB 1-10		Vegetative Protection LDB 1-10	
	Vegetative Protection RDB 1-10		Vegetative Protection RDB 1-10	
10	Riparian Zone LDB 1-10		Riparian Zone LDB 1-10	
	Riparian Zone RDB 1-10		Riparian Zone RDB 1-10	
	Total	_____	Total	_____

Appendix D (i)

Benthic Macroinvertebrate Field Data Sheet (back)

Weather observations

Current weather:_____

Recent precipitation:_____

Stream flow (circle one) Low Normal Above Normal Flood

Biological Observations

circle one in each category

0 1 2 3	Periphyton	**0 1 2 3**	Salamanders
0 1 2 3	Filamentous algae	**0 1 2 3**	Warmwater Fish
0 1 2 3	Submerged Macrophytes	**0 1 2 3**	Coldwater Fish
0 1 2 3	Emergent Macrophytes	**0 1 2 3**	Beavers
0 1 2 3	Crayfish	**0 1 2 3**	Muskrats
0 1 2 3	Corbicula	**0 1 2 3**	Ducks/Geese
0 1 2 3	Unionidae	**0 1 2 3**	Other…
0 1 2 3	Operculate Snails	**0 1 2 3**	
0 1 2 3	Non-operculate Snails	**0 1 2 3**	
0 1 2 3	Frogs/ Tadpoles	**0 1 2 3**	

0 = Absent
1 = Sparse
2 = Common to Abundant
3 = Dominant - abnormally high density where other taxa are insignificant in relation to the dominant taxa. There can be situations where multiple taxa are dominant such algae and snails.

Notes: _____

Appendix D (ii)

Benthic Macroinvertebrate Laboratory Bench Sheet

Station ID: _____ Sample subsorted by: _____ Date: _____

Stream Name: _____

Date Sampled: _____ Sample Identified by: _____ Date: _____

Sampling Method: _____

Taxa Collected:

Porifera
- Spongillidae_____

Flatworms
- Dendrocoelidae_____
- Planariidae_____

Limpets
- Ancylidae_____

Snails
- Lymnaeidae_____
- Physidae_____
- Planorbidae_____
- Hydrobiidae_____
- Pleuroceridae_____
- Viviparidae_____

Unionida
- Corbiculidae_____
- Sphaeriidae_____
- Unionidae_____

Lumbriculida
- Lumbriculidae_____

Tubificida
- Enchytraeidae_____
- Naididae_____
- Tubificidae_____

Haplotaxida
- Haplotaxidae_____

Leeches
- Erpobdellidae_____
- Glossiphoniidae_____
- Hirudinidae_____
- Pisciolidae_____

Branchiobdellida
- Branchiobdellidae_____

Decapoda
- Cambaridae_____

Shrimp
- Palaemonidae_____

Isopoda
- Asellidae_____

Amphipoda
- Gammaridae_____
- Talitridae_____

Water Mites
- Hydracarina_____

Ephemeroptera
- Ameletidae_____
- Baetidae_____
- Baetiscidae_____
- Caenidae_____
- Ephemeridae_____
- Heptageniidae_____
- Isonychiidae_____
- Leptophlebiidae_____
- Neoephemeridae_____
- Polymitarcyidae_____
- Potamanthidae_____
- Siphlonuridae_____
- Tricorythidae_____

Zygoptera
- Calopterygidae_____
- Coenagrionidae_____
- Ephemerellidae_____
- Lestidae_____
- Protoneuridae_____

Anisoptera
- Aeshnidae_____
- Cordulegastridae_____
- Corduliidae_____
- Gomphidae_____
- Libellulidae_____
- Macromiidae_____
- Petaluridae_____

Plecoptera
- Capniidae_____
- Chloroperlidae_____
- Leuctridae_____
- Nemouridae_____
- Peltoperlidae_____
- Perlidae_____
- Perlodidae_____
- Pteronarcyidae_____
- Taeniopterygidae_____

Hemiptera
- Belostomatidae_____
- Corixidae_____
- Gelastocoridae_____
- Gerridae_____
- Hebridae_____
- Hydrometridae_____
- Mesoveliidae_____
- Naucoridae_____
- Nepidae_____
- Notonectidae_____
- Veliidae_____

Neuroptera
- Sisyridae_____

Megaloptera
- Corydalidae_____
- Sialidae_____

Trichoptera
- Brachycentridae_____
- Calamoceratidae_____
- Glossosomatidae_____
- Helicopsychidae_____
- Hydropsychidae_____
- Hydroptilidae_____
- Lepidostomatidae_____
- Leptoceridae_____
- Limnephilidae_____
- Molannidae_____
- Odontoceridae_____
- Philopotamidae_____
- Phryganeidae_____
- Polycentropodidae_____
- Psychomyiidae_____
- Rhyacophilidae_____
- Sericostomatidae_____
- Uenoidae_____

Lepidoptera
- Pyralidae_____

Coleoptera
- Chrysomelidae_____
- Curculionidae_____
- Dryopidae_____
- Dytiscidae_____
- Elmidae_____
- Gyrinidae_____
- Haliplidae_____
- Helodidae_____
- Hydrophilidae_____
- Limnichidae_____
- Noteridae_____
- Psephenidae_____
- Ptilodactylidae_____

Diptera
- Athericidae_____
- Blephariceridae_____
- Canaceidae_____
- Ceratopogonidae_____
- Chaoboridae_____
- Chironomidae (A)_____
- Chironomidae (B)_____
- Culicidae_____
- Dixidae_____
- Dolichopodidae_____
- Empididae_____
- Ephydridae_____
- Muscidae_____
- Psychodidae_____
- Ptychopteridae_____
- Sciomyzidae_____
- Simuliidae_____
- Stratiomyidae_____
- Syrphidae_____
- Tabanidae_____
- Tanyderidae_____
- Tipulidae_____

TOTAL: _____

Use back of sheet for subsampling information

Appendix E

Virginia Department of Environmental Quality
Biological Monitoring Program
305(b) Assessment Fact Sheet

Regional Office:
Regional Biologist's Signature: _____
Review Date:
River Basin:
Stream Name and Site Location:
Station ID #:
Reference Station ID #:
Assessment Method:
EPA RBP-II
Coastal Plain

Biological Assessments for the Last Five Years

Year	spring score	Spring assessment	Fall score	fall assessment
2000				
2001				
2002				
2003				
2004	0.0		0.0	
Seasonal avg 5-yrs	0.0		0.0	
Seasonal avg last 2-yrs	0.0		0.0	
Final 5-yr average	0.0		0.0	
Final 2-yr average	0.0		0.0	

Note, because of the long, five-year time frame covered by this review and for a variety of reasons, some sites may not have been sampled during every year or season and/or an assessment ranking or score may not be available for every "cell" in the above table. The above table is intended to be a convenient method to summarize and review all the data available for the reporting period. The final assessment ranking for each site should be based on a review of all the available rankings shown in the above table and any pertinent supplemental data described below. For the purpose of 305(b) report preparation, if more recent bioassessment rankings differ significantly from earlier rankings, primary consideration should be given to the more recent assessment data. This is described in more detail of section 6.4.1 of the 305(b) Guidance Manual.

Supplemental Information (if applicable):

Are any seasonal differences noted?

Summary of any comments associated with assessments.

Have any factors been observed in watershed that may be affecting the benthic community? Have there been any recent changes in activity in the watershed that may have affected the more recent bioassessments. Are these changes likely to affect the benthic community for a short or long term basis?

Final Assessment Rating: